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MMed Part III (minor dissertation)

Retrospective Study of Patients Treated for Plasmablastic Lymphoma at Groote Schuur Hospital between 2004 and 2009

by

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DECLARATION

I Dr Sebathu Phillip Chiyapo declare that the work on this study is originally my work except where acknowledgements are indicated. This is an unsponsored study and was carried out for educational purposes only as a commentary for a postgraduate degree. I therefore declare no conflict of interest whatsoever.

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PART A: ABSTRACT AND STUDY PROTOCOL

ABSTRACT

Objective: The purpose of this study is to evaluate the demographics, disease profile and treatment outcome of patients treated at Groote Schuur Hospital in Cape Town for Plasmablastic Lymphoma over a 5 year period extending from 2004 to 2009.

Background: Plasmablastic lymphoma (PBL) is a highly aggressive rare subtype of Diffuse Large B-Cell Lymphoma first described in 1997. The diagnosis of PBL remains a challenge despite advances in diagnostic tools. There is no established standard of care, although CHOP chemotherapy is an accepted choice of treatment. The prognosis is poor even with more intense chemotherapy regimens.

Methods: Medical records of 28 patients treated for PBL between January 2004 and December 2009 were reviewed. Factors evaluated for the impact on overall survival (OS) included stage, IPI, CD4 count, chemotherapy alone vs. chemotherapy and radiotherapy, extranodal status and gender. Kaplan –Meier methods were used to compare patient outcomes including the 1 and 3 year survival proportions respectively.

Results: There were 25 HIV positive patients. The median age at presentation for the HIV positive group was 35 years (30-57) with a slight female predominance. Distribution of population by race was 80% blacks, 16% mixed race and 4% whites. CHOP chemotherapy was used as the primary treatment in our institution and patients with early stage disease received radiotherapy after chemotherapy. The objective overall response rate was 68% for HIV positive patients with a median OS of 52 weeks. Factors associated with good survival outcome were early stage disease, combination of chemotherapy with radiotherapy and low IPI score. The one and three year OS were 45% and 39% respectively with a relapse rate on average of 17%.

Conclusion: To date the survival outcome for PBL remains very poor regardless of intervention. In this study the primary treatment was CHOP chemotherapy which was combined with radiotherapy when indicated. The survival outcomes in this study are comparable to previously published studies but with a superior proportion of surviving patients 5 years post treatment. Good prognosis is associated with early stage disease and treatment with combination of chemotherapy and radiotherapy.

Key Words: Plasmablastic Lymphoma, HIV, overall survival, CHOP chemotherapy, PBL, prognostic factors, IPI (International Prognostic Index)

MMED STUDY PROTOCOL

Tittle of Project

Retrospective Study of Patients Treated for plasmablastic lymphoma(PBL) at Groote Schuur Hospital between 2004 and 2009

Research Question

What are the treatment outcomes, disease profiles and demographics of a group of patients treated for plasmablastic lymphoma in Groote Schuur Hospital over a five year period ranging from 2004 to 2009?

Purpose of Study

The purpose of this study is to retrospectively evaluate the treatment outcomes, disease profiles and demographics of patients with plasmablastic lymphoma.

Patient Population

This study included all patients over the age of 18 with a confirmed histological diagnosis of plasmablastic lymphoma treated at Groote Schuur Hospital between 2004 and 2009.

Background

Plasmablastic lymphoma is a highly aggressive, rare subtype of Diffuse Large B-Cell Lymphoma. This condition was first described by Delecluse et al in 1997[1]. It is widely described in HIV positive patients and accounts for 2.6% of all HIV related Non-Hodgkin lymphomas [2]. This tumour mostly occurs in the oral cavity. It is most common in the middle aged group ranging from 35-55 years[3] but it has been reported in the paediatric population. There is not a lot of data available in the literature. A few cases have been reported in HIV negative patients with most of them having immunosuppressive conditions which include transplant patients on immunosuppressive drugs or chronic steroid use, Crohn's disease and also seen in immunocompetent patients[4-9]. Most reported cases are associated with Epstein-Barr virus (EBV) and the role of Human Herpes Virus 8(HHV8) is not yet clear[10, 11].

The incidence has increased following the introduction of HAART. These lymphomas are thought to be frequently resistant to therapy with a poor prognosis, high relapse rates and

death occurring 1 to 24 months after diagnosis. There are currently no clear and validated treatment guidelines. CHOP chemotherapy is widely accepted as first line of treatment and variable treatment options are available for relapsed disease [12].

This study will be among the larger cohorts done on this topic and the information obtained may be useful in the management of this rare malignancy locally and worldwide.

Objective

The primary objective of this review is to evaluate the survival outcomes of plasmablastic lymphoma patients treated at our institution between 2004 and 2009 with the following endpoints:

- Overall survival
- Patient demographics
- Relapse rates

The other objectives will be to evaluate the influence of:

- Tumour related factors including disease site, stage, B-symptoms, performance status, lactate dehydrogenase and International Prognostic score
- HIV related factors including whether on HAART at lymphoma diagnosis, CD4 count at diagnosis, opportunistic infections or presence of any other malignancies.

Methods and Data Collection

Folders for patients treated for PBL between 2004 and 2009 were retrospectively reviewed. The list for these patients was obtained from the Groote Schuur Hospital Radiation Oncology Department. Ethics approval was obtained from the Health and Research Ethics Committee of the University of Cape Town and permission from the Clinical Director of Groote Schuur Hospital was obtained before commencement of the study. A literature review was performed using PubMed, Google, available journals and text books.

All folders reviewed included only patients over the age of 18 years with a confirmed histological diagnosis of PBL. The data extracted was recorded into customised data sheets and transferred into an excel spreadsheet.

The data collected included age at diagnosis, date of presentation, gender, ethnic group, site of disease, stage of disease, ECOG performance status, Lactate Dehydrogenase (LDH) and the International Prognostic Index (IPI). The IPI was calculated by incorporating the following factors; age >60 years, serum Lactate dehydrogenase (LDH) levels >1x normal, ECOG performance status of 2-4, stage III or IV and >1 extranodal site involvement. The presence of B-symptoms which included weight loss of >10% in the last 6 months, unexplained fever or night sweats was noted.

In addition to the above, HIV status, CD4 count and viral load when available were included in the data sheet as well as the presence of any infections like pulmonary tuberculosis (PTB) and varicella zoster, and other malignancies like Kaposi sarcoma. Time on HAART at diagnosis was also recorded

The treatment approach was documented including the chemotherapy regimen used, number of cycles completed and radiotherapy doses for those patients who received radiotherapy. Responses to treatment were recorded as complete response (CR), partial response (PR) and progressive disease (PD). For patients who achieved a complete response and relapsed, the time to relapse, site of relapse and the treatment offered for the relapse was documented.

At last follow up, the patients' status was recorded as either alive with disease, alive without disease or dead, and if recorded as dead it was noted whether they died from lymphoma or other causes.

Statistical analysis was performed using GraphPad Prism version 6 for Windows. Overall Survival (OS) was calculated from time of registration to time of death due to lymphoma or any other cause or date last seen for patients lost to follow up. Progression Free Survival (PFS) was calculated from time of registration to time of first progression on primary treatment, death or date last seen for those patients lost to follow up. Survival curves were generated using the Kaplan-Meier method and compared using the log rank test. A p value of <0.05 was considered statistically significant.

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PART B: STRUCTURED LITERATURE REVIEW

Objectives of literature review

The objective of this literature review is to provide the reader with background information on the topic in order to justify this study and present research done by other authors in the field. This will allow data comparison, identify new evidence in the literature and recognise areas that require further research.

Literature Search strategy

For this study, articles relating to plasmablastic lymphoma were searched for using the following search engines; PubMed, Google, Google scholar, read by QXMD app, text books and journal articles. These studies were published from 1997 after the first article describing plasmablastic lymphoma was published. Although plasmablastic lymphoma was the main search term used, other HIV related lymphomas and B-cell lymphomas were also included in the search as these conditions are clinically similar. Only publications in the English language were included in this review.

In the last decade several case reports have been published exploring the pathogenesis, clinical behaviour, treatment options and outcomes, influence of HAART and prognostication of plasmablastic lymphoma. These are mostly case reports and single institution retrospective studies. There are no published meta-analyses or phase III studies, which in clinical practice are believed to confer a higher level of evidence with regard to plasmablastic lymphoma.

Interpretation of literature

In order to understand and discuss the results of the current study, a detailed review of the available literature was performed and discussed. This review will summarise plasmablastic lymphoma as follows:

a) Background information

b) Epidemiology

c) Pathogenesis

d) Pathological features

e) Clinical presentation

f) Treatment and outcomes

g) Prognosis

h) Conclusion

a) Background information

Plasmablastic lymphoma is a highly aggressive, rare subtype of diffuse large B-cell lymphoma (DLBCL). This condition was first described by Delecluse and colleagues in 1997. The subjects described by Delecluse all presented with oral cavity involvement and the majority were HIV positive [1]. Recently, the literature has reported a more heterogeneous involvement by site [2, 3].

Although plasmablastic lymphomas were initially reported in HIV positive adults, emerging literature describes this malignancy in immunocompetent individuals with no known medical history [4, 5]. However, some HIV negative patients reported on were immunosuppressed due to drugs like chronic steroids for autoimmune conditions, chronic viral infection [3] or in the post-transplant setting [2, 6]. Plasmablastic lymphoma has been reported in the paediatric population both in the setting of HIV infection and in immunocompetent children [7, 8].

Over the last decade and a half, several reports have emerged describing plasmablastic lymphoma. These have been small reviews including single institutional case studies and retrospective studies. The classification of plasmablastic lymphoma has changed quite significantly in the last decade and a half. In 2001, the WHO classified plasmablastic lymphoma as a lymphoma of the oral cavity occurring more specifically in people who are HIV positive. This nomenclature was chosen due to the predominant occurrence in the oral cavity. In 2008, the WHO classified plasmablastic lymphoma as a separate category under aggressive B-cell lymphomas and the nomenclature is no longer restricted to the oral cavity since extraoral occurrences have been widely described [9].

This malignancy presents a dilemma in that the pathogenesis, clinical presentation and treatment outcomes are poorly understood. There is no established standard of care therapy for primary or relapsed disease. Despite the treatment approach used, response rates can be high but relapse rates are also high and death can occur within one month to two years after diagnosis [10]. In the setting of HIV infection the introduction of highly active anti-retroviral therapy (HAART) has led to improved overall survival rates and response rates in patients with AIDS related lymphomas but studies have shown conflicting results since the incidence of AIDS related lymphomas has changed with the introduction of HAART [11-14].

This malignancy is believed to carry a poor prognosis; however several studies have failed to establish prognostic factors associated with plasmablastic lymphoma. Several studies with conflicting results have been published on prognostication of plasmablastic lymphoma [4, 10, 15]. The emergence of aggressive AIDS related lymphomas like plasmablastic lymphoma, Burkitt's lymphoma and primary effusion lymphoma pose a health challenge in the Sub-Saharan region where there are limited resources allocated to health care.

In South Africa there is a paucity of data available on AIDS-related malignancies. With the high incidence of HIV in this setting as evidenced by the Midyear Population Estimates 2011 report by Statistics South Africa [16], an increased incidence of AIDS related lymphomas including plasmablastic lymphoma has been reported [13].

b) Epidemiology

Plasmablastic lymphoma is a rare malignancy. The true incidence is unknown but it is estimated to make up to 2.6% of all HIV related Non-Hodgkin lymphomas [17]. The majority of patients are in the 35-55 age population group and the incidence is higher in males [18, 19].

The majority of cases are associated with HIV infection. The demographics of this disease are unclear but it has been reported in various populations from across different continents and countries [11, 20-22]. In a study by Abayomi et al, they noted an increase in the incidence of AIDS related lymphomas in the Western Cape in South Africa over an eight year period and the emergence of new entities like plasmablastic lymphoma. They also noted that the roll out of HAART in 2004 did not appear to have had any impact on the incidence of AIDS related lymphomas in this region thus far [13].

Wiggill and her colleagues in a study performed in Johannesburg, South Africa demonstrated an increase in the incidence of lymphoproliferative malignancies in the HIV positive population from 44.3% between 2004 and 2006 to 62% between 2006 to 2009 [23]. The same study also showed a decrease in the age at presentation from a median age of 47 in the HIV negative to 36 in the HIV positive and the male to female ratio. Several other studies have reported similar trends of increased incidence in areas with high HIV prevalence [20, 24, 25]

c) Pathogenesis

The available pathological, immunohistochemical, molecular and genetic data suggests that plasmablastic lymphoma arises from post-germinal centre, terminally differentiated, activated B-cells in transition from immunoblasts to plasma cells [26].

Epstein - Barr virus (EBV) has been found to be associated with AIDS related lymphomas and is thought to play a key role in its pathogenesis. In one series there was a 70% association between plasmablastic lymphoma and EBV [10, 27-29]. EBV is a gamma herpes virus implicated in the aetiology of malignancies like Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma and HIV-related lymphomas. The role played by EBV in the pathogenesis of plasmablastic lymphoma is being explored. Carbone and colleagues postulated that EBV encodes a series of products interacting with a wide variety of antiapoptotic molecules, cytokines and signal transducers thereby promoting EBV infection, cell immortalization and transformation to malignant cells and also has the capacity to induce unlimited proliferation of B-lymphocytes by in vitro transformation [27, 30].

In a study by Qing and colleagues, a case of plasmablastic lymphoma occurring as a result of transformation from plasmacytoma was reported [31]. This was reported by others who demonstrated the immunophenotypic similarities between plasmablastic lymphomas and plasma cell myelomas [6]. These findings were however refuted by studies demonstrating conflicting results. Chung Che and colleagues reported more similarities with DLBCL than plasma cell myelomas [32].

Human Herpes Virus 8 (HHV8), another member of the herpes virus family, has been associated with the pathogenesis of other AIDS related malignancies like Kaposi's sarcoma and Castleman's disease. It does not appear to play a role in the pathogenesis of plasmablastic

lymphoma, except in documented cases of plasmablastic lymphoma arising in HHV8 associated Multicentric Castleman's disease [33, 34]. This type of plasmablastic lymphoma is considered a separate entity by most authors [35 36].

Plasmablastic lymphoma is associated with rearrangements in the c-myc gene in more than 40% of cases [36, 37]. Valera and colleagues reported c-myc rearrangement in 50% of cases reviewed and noted that EBV positive patients were more likely to have the c-myc abnormality. Conversely, c-myc rearrangement occurred less frequently in EBV negative patients [38]. This mutation is associated with very aggressive AIDS related lymphomas like Burkitt's lymphoma [39].

Other theories of pathogenesis have been postulated. Martinez and colleagues reported six cases of plasmablastic differentiation as a transformation from low grade B-cell lymphomas including three cases from chronic lymphocytic leukaemia and three from follicular lymphoma [37, 40]

The pathogenesis of this malignancy is an active area of research. Understanding the pathogenesis may guide the management and lead to improved outcomes for patients with plasmablastic lymphoma.

d) Pathological features

Plasmablastic lymphomas share morphological, pathological and immunohistochemical characteristics with other types of lymphomas notably diffuse large B-cell lymphoma, Burkitt's lymphoma and plasma cell myelomas. Chung Che and colleagues reported more similarities between plasmablastic lymphoma and diffuse large B-cell lymphoma than with plasma cell myelomas [32]. These results differed from the findings of Qing and colleagues who described a picture more similar to plasma cell myelomas than diffuse large B-cell lymphomas [31]. These similarities can make the diagnosis difficult and adversely affect the management of plasmablastic lymphoma.

Although Plasmablastic lymphomas are described as aggressive B-cell lymphomas they lack B-cell markers like CD45, CD79a, PAX5 and have little or no expression of CD20 [10, 41].

CD20 expression was found to be higher in the HIV positive group compared to the HIV negative group in a study by Castillo and colleagues [4]. These malignancies express plasma cell markers like CD38, CD138, VS38C and MUM1 (multiple myeloma oncogene-1).

Immunohistochemistry is essential in the diagnosis of Plasmablastic lymphoma. In the resource-limited Sub-Saharan setting the diagnosis of Plasmablastic lymphoma presents a challenge as immunohistochemistry is not always available. The newer stains used in the identification of Plasmablastic lymphoma like PRDM1/BLIMP1 protein and XBP1 proteins [42] are not cost effective in this setting.

Kane and colleagues, working at a cancer institute in India proposed that the minimum immunohistochemical tests required to be able to diagnose plasmablastic lymphoma includes the CD20, CD138 and CD38 stains as well as a ki-67>60% and expression of EBV[43]. These may not be available at all laboratories in the developing world.

FISH studies have demonstrated myc/IgH mutations which are also present in Burkitt's lymphoma and found to a lesser extent in aggressive B-cell lymphomas [38]. These genetic aberrations are associated with a more aggressive course and worse treatment outcome.

e) Clinical presentation

In the initial studies, plasmablastic lymphoma was reported in HIV positive patients and mostly described in the oral cavity [1, 44]. This malignancy has now been found to occur in the HIV negative population as well. Castillo and colleagues described 76 HIV negative patients with plasmablastic lymphoma. They concluded that plasmablastic lymphoma in the HIV negative population had a striking predilection for extraoral sites and had less association with EBV than in the HIV positive group. Overall survival was shorter in the HIV negative cohort [4]. Similar results were reported by Liu and colleagues, who also reported a higher incidence of women and a higher median age at presentation in the HIV negative population [45]. At present it is unclear if the introduction of HAART has modified these factors.

Both HIV positive and HIV negative plasmablastic lymphoma have been shown to involve multiple heterogeneous sites. Other sites reported include the larynx [46], central nervous system, gastrointestinal system, kidneys and skin [2, 4, 5, 47]. Extranodal involvement occurs

much more frequently than nodal involvement in plasmablastic lymphoma [10]. It is unclear whether oral and extraoral plasmablastic lymphomas are the same clinical entities.

Hansra and colleagues reported that patients with oral involvement had a better overall survival than those with extraoral plasmablastic lymphoma. This may indicate that these are two different clinicopathologic entities [48]. Plasmablastic lymphoma can be the initial presentation in patients with previously unknown HIV status [49] or can present in patients known to be HIV positive on HAART. In patients known to be HIV positive the average CD4 count at presentation with lymphoma is 178 cells/mm3 and the average viral load is 86,000 copies/mL from a study by Castillo and colleagues, B symptoms including more than 10% weight loss, unexplained fever or night sweats has been shown to be more common in the HIV negative than the HIV positive population. The same study also shown LDH is elevated in the majority of patients at presentation particularly in those who present with advanced stage i.e. stage III and IV according to the modified Ann Arbor staging [10].

f) Treatment and treatment response.

Plasmablastic lymphomas are thought to be resistant to standard Non-Hodgkin's lymphoma therapy with poorer prognosis and death occurring within a months after diagnosis. There is no established standard of care or validated treatment protocols for plasmablastic lymphoma. Cyclophosphamide, Oncovin (vincristine), Adriamycin (hydroxydaunorubicin) and Prednisone (CHOP) chemotherapy regimen and CHOP like regimens have been used worldwide and is accepted as first line treatment [10]. The NCCN however recommends more aggressive and intense treatment approaches like CODOX/IVAC (cyclophosphamide, vincristine, adriamycin, methotrexate, ifosfamide, etoposide and cytarabine), dose adjusted EPOCH (etoposide, adriamycin, vincristine, cyclophosphamide and prednisone) and HyperCVAD (cyclophosphamide, vincristine, adriamycin, dexamethasone, methotrexate and cytarabine) [50]. The NCCN guidelines state that CHOP is an inadequate treatment. This is based on evidence from case reports only since there are no randomised clinical trials in this regard.

With CHOP and CHOP like regimens high objective response rates have been achieved. Castillo and colleagues described complete response rates of 46% and partial response rates

of 31%. In the same study 72% of patient's progressed and 57% were deceased at the time of publication. The overall survival achieved in the same study was 31% at 5 years with a median survival of 14 months [15]. These results were echoed by other studies [4, 10, 51]. Long term survival is a rare phenomenon in plasmablastic lymphoma. Sharma and colleagues report on a patient still alive eight years post treatment [52] which appears to be the longest survivor in the reviewed literature.

With the introduction of HAART improved overall survival rates and response rates have been observed [21, 53, 54]. A few cases of spontaneous regression have been documented with the use of HAART only [55, 56].

Bortezomib, a proteasome inhibitor effective in the management of multiple myeloma has been studied in patients with plasmablastic lymphoma. Bibas and colleagues used bortezomib alone or in combination with dexamethasone, oxaliplatin, gemcitabine, cytarabine and pegfilgrastin (GOVDD regimen). They reported that the results were promising but they failed to show any survival advantage over standard chemotherapy [57].

Rituximab, an anti-CD20 monoclonal antibody, is widely used as the standard of care in combination with CHOP chemotherapy for Diffuse Large B-cell lymphoma. Occasionally there is weak expression of CD20 in some cases of Plasmablastic lymphoma [10] and therefore the use of rituximab has been suggested in this group.

Castillo and colleagues evaluated treatment outcomes in patients receiving CHOP, CHOP-like regimens and more intense regimens. They reported no statistical difference in the overall survival between the less and more intensive treatment regimens [15, 58].

High dose chemotherapy and stem cell transplant is a well documented treatment for DLBCL at relapse, and other highly aggressive lymphomas in first remission. Its role and effectiveness in the management of plasmablastic lymphoma has not been tested but some authors suggest that it may be a viable option for use at first remission [36, 58, 58].

Radiotherapy has also been used in the treatment or in combination with chemotherapy with or without HAART with inconclusive results [10, 15]. It is generally used as palliative therapy.

In cases of relapsed or refractory disease the data concerning further management is scanty. Palliative chemotherapy and radiotherapy or combinations of the two are viable treatment options, but due to high mortality rates there is limited data available to substantiate its use.

g) Prognosis

A few studies have investigated possible factors influencing prognosis in plasmablastic lymphoma. In a study by Castillo et al early clinical stage and complete response to chemotherapy were associated with improved survival rates [15]. In another study with 112 reported cases (oral and extra oral) 66% achieved a complete response and 29% progressed. In the same study 25% of patients relapsed and 53% died with majority of them dying from lymphoma. Oral cavity site and stage I disease was associated with better survival outcomes and the following factors failed to show any association with overall survival: gender, CD4 count, viral load, chemotherapy used and EBV status [10].

HIV negative patients with plasmablastic lymphoma had a poorer overall survival than HIV-positive patients [45].

The presence of a MYC/IgH rearrangement was associated with a worse overall survival [15, 36, 38]. This gene rearrangement is common in Burkitt's lymphoma which is known to be a very aggressive B-cell lymphoma [39].

The use of HAART has also been associated with better survival outcomes [15].

Conclusion

The treatment of plasmablastic lymphoma should be approached in a multidisciplinary fashion. The overwhelming majority of these patients are HIV positive and the burden of disease appears to be greater in regions with a high incidence of HIV. HIV/AIDS has resulted in an increased economic burden on health systems in both developing and developed countries with the developing world bearing the brunt of this disease due to poor resources and infrastructure combined with large patient numbers.

There are no large randomised trials for the management of plasmablastic lymphoma. The rarity of this disease poses a unique challenge to clinicians in identifying patients at risk, making the diagnosis and treating the disease. As evidenced by this literature review, plasmablastic lymphoma has unique clinicopathological characteristics yet shares many clinical, pathological and immunohistochemical properties with other B-cell and plasma cell malignancies.

In the absence of established standard of care guidelines the ideal management of these patients would be in a clinical trial setting. Currently treatment is individualised according to the limited evidence and resources available to the treating institution. The integration of HAART with chemotherapy as well as the management of opportunistic infections is very important; therefore it is essential to work as a multidisciplinary team with the infectious diseases specialists. Immunotherapy, radiotherapy and stem cell transplant can be used as per the treating physicians' discretion and available resources.

In the Sub-Saharan setting where the incidence of HIV/AIDS and consequently aggressive HIV associated lymphomas are high, conducting multinational randomised clinical trials will contribute to the knowledge base which may help in the future management of patients with plasmablastic lymphomas and other aggressive B-cell lymphomas. It is vitally important to understand the pathogenesis of this disease in order to manage it effectively. In conclusion, in the HIV-positive population this is a disease of severely immunosuppressed individuals. Optimising HIV management and improving access to antiretroviral treatment should decrease the incidence of this disease and improve treatment outcomes.

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PART C: PUBLICATION-READY MANUSCRIPT

PART C: PUBLICATION-READY MANUSCRIPT - TITLE PAGE.

"Retrospective Study of Patients Treated for Plasmablastic Lymphoma at Groote Schuur Hospital between 2004 and 2009".

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ABSTRACT

Background: Plasmablastic lymphoma (PBL) is a highly aggressive rare subtype of diffuse large B-cell lymphoma first described in 1997. The diagnosis of PBL remains a challenge despite advances in diagnostic tools. There is no established standard of care, although CHOP chemotherapy is an accepted choice of treatment. The prognosis is poor even with more intense chemotherapy regimens. The purpose of this study is to evaluate the demographics, disease profile and treatment outcome of patients treated at Groote Schuur Hospital in Cape Town for Plasmablastic Lymphoma over a 5 year period extending from 2004 to 2009.

Methods: Medical records of 28 patients treated for PBL between January 2004 and December 2009 were reviewed. Factors evaluated for the impact on overall survival (OS) included stage, IPI, CD4 count, chemotherapy alone vs. chemotherapy and radiotherapy, extranodal status and gender. Kaplan –Meier methods were used to compare patient outcomes including the 1 and 3 year survival proportions respectively.

Results: There were 25 HIV positive patients. The median age at presentation was 35 years (30-57) with a slight female predominance. Distribution of population by race was 80 % blacks, 16% mixed race and 4% whites. CHOP chemotherapy was used as the primary treatment in this institution and patients with early stage diseases received radiotherapy after chemotherapy. The objective overall response rate was 68% for HIV positive patients with a median OS of 52 weeks. Factors associated with good survival outcome were early stage disease, combination of chemotherapy with radiotherapy and low IPI score. The one and three years OS were 45% and 39% respectively with a relapse rate of 17% at 68 weeks on average.

Conclusion: To date the survival outcome for PBL remains very poor regardless of intervention. In this study the primary treatment was CHOP chemotherapy which was combined with radiotherapy when indicated. The survival outcomes in this study were comparable to other studies but with a superior proportion of surviving patients 5 years post

treatment. Good prognosis is associated with early stage disease and treatment with a combination of chemotherapy and radiotherapy.

Key Words: Plasmablastic Lymphoma, HIV, overall survival, CHOP chemotherapy, PBL, prognostic factors, IPI (International Prognostic Index)

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Background

Plasmablastic lymphoma (PBL) is a highly aggressive, rare subtype of Diffuse Large B-Cell Lymphoma (DLBCL). This condition was first described by Delecluse et al in 1997 [1]. It is widely described in HIV positive patients and accounts for 2.6% of all HIV related Non-Hodgkin lymphoma. It was first described in the oral cavity but it is now known to occur in other sites [2, 3].

It occurs mostly in the middle aged population group ranging from 35-55 years [4,5]. In the 2001 WHO classification, plasmablastic lymphoma of the oral cavity was classified in the category of lymphomas occurring more specifically in people who are HIV positive. According to the 2008 WHO classification it is now a separate category under aggressive B-cell lymphomas [6]. This tumour has also been reported in the paediatric population with a few case reports documented for immunocompetent children and HIV positive children as well as in paediatric and adult patients in the post-transplant setting [7-10].

There is limited data available in the literature on this malignancy. A few cases have been reported in HIV negative patients but most of these were immunosuppressed due to immunosuppressive drugs used post-transplant or chronic steroids use for autoimmune conditions like Crohn's disease [9,11,12]. A few cases have been reported in

immunocompetent patients with no history of any known medical conditions [13]. In addition most reported cases are associated with Epstein-Barr virus (EBV). The role of Human Herpes Virus 8 (HHV8) is not clear except in documented PBL arising in HHV-8 associated Multicentric Castleman's disease [14,15].

The pathogenesis of PBL is poorly understood. From the available clinical, immunohistochemical, molecular and genetic studies it is thought that PBL arises from post-germinal centre, terminally differentiated, activated B-cells in transition from immunoblast to plasma cells [16]. PBL has been shown to be morphologically and immunohistochemically similar to plasmablastic plasma cell myeloma (PCM) by expression of plasma markers CD38, CD138, VS38C, MUM1 and lack of B-cell markers like CD20. Recent studies have also shown that c-myc gene rearrangement which is common in Burkitt's lymphoma may also play a role in more than 40% of cases [2,16-19].

These lymphomas are frequently resistant to therapy with poorer prognosis and death occurring within months after diagnosis. The introduction of HAART has led to patients with AIDS related lymphomas (ARL) experiencing improved survival rates and overall response rates. Studies have shown conflicting results in establishing clearly if the incidence of ARL's has changed with the introduction of HAART [20-24]. There are no clear and validated treatment guidelines for plasmablastic lymphoma. The CHOP (Cyclophosphamide, Adriamycin, Vincristine and Prednisone) chemotherapy regimen is widely accepted as first line of treatment, however the NCCN guidelines recommends more aggressive regimens like CODOX-M/IVAC (cyclophosphamide, vincristine, adriamycin, methotrexate, ifosfamide, etoposide and cytarabine) regimen, dose adjusted EPOCH (etoposide, adriamycin, vincristine, cyclophosphamide and prednisone) and HyperCVAD (cyclophosphamide, vincristine, adriamycin, dexamethasone, methotrexate and cytarabine) [25]. Treatment options available for relapsed or progressive disease include palliative chemotherapy, palliative radiotherapy or a combination of these two treatment modalities but the outcomes of these options at relapse or progression are lacking.

The purpose of this study was to evaluate the treatment outcomes of patients treated for PBL between 2004 and 2009 by evaluating the overall survival, response rates, disease free survival and patient demographics. The study was also aimed at evaluating tumour and patient factors and how they affect treatment outcomes.

Results

Between January 2004 and December 2009, 28 patients were treated for plasmablastic lymphoma at Groote Schuur Hospital. The patients profile and disease characteristics is shown in Table 1. There were 25 (89.3%) HIV positive and 3 (10.7%) HIV negative patients. The median age at presentation for all patients was 35 years (range 24-57) and the median follow up for all patients was 36 weeks.

HIV Negative patients

For the three HIV negative patients: one patient died before treatment was commenced, one patient died after one cycle of CHOP and the last patient progressed after five cycles of CHOP, then received one cycle of DHAP with progression of disease. He had palliative radiotherapy but died shortly after. These patients had no other known medical conditions or immunosuppressive conditions. They were aged 24, 34 and 48 and involved sites included mediastinum, brain and oral cavity. Further detailed analysis of this group of patients will not be described in this audit due to the small sample size.

HIV Positive patients

The median age at presentation was 35 (range 30-57). The median follow up was 36 weeks (range 3-292). There were 12 male and 13 female patients at a ratio of 1:1.08. Black patients accounted for 80%, mixed race patients 16% and there was one white patient (4%).

At presentation 52% of HIV positive patients had early stage disease (stage I/II). The majority of these patients (72%) presented with extranodal disease only, 4% had only nodal involvement and the rest of group (16%) had involvement of both nodal and extranodal sites. In patients with extranodal disease, 63% had only one extranodal site involved whereas 37% had more than one extranodal site involved. The distribution of disease by site is shown in

Table 2. Bone marrow involvement occurred in three patients. The most common extranodal sites involved were bone (21%), oral cavity and oropharynx (18%), nasopharynx and paranasal sinuses (18%), anorectal area and spleen at 14% per site.

According to the International Prognostic Index, approximately half of patients presented with low risk disease, about a third presented with intermediate risk disease (low intermediate and high intermediate disease were considered as one group) and only 8% of patients presented with high risk disease. B-symptoms were experienced by 32% of patients at presentation. The median CD4 count at presentation was 196 cells/ml^3 (range 23 cells/ml^3 to 368cells/ml^3) and viral load was known in only three patients at presentation ranging from 890 copies/ml- 70000 copies/ml. At the time of presentation, only eight (32%) patients were on HAART and median time on HAART was 10 weeks (range 1-24 weeks). PBL was the initial manifestation of HIV/AIDS in 68% of patients whose HIV status was previously unknown prior to PBL diagnosis. At least 13 (52%) patients were on treatment for tuberculosis or had a history of tuberculosis treatment. Kaposi sarcoma was diagnosed in two of the 25 HIV positive patients who presented to our clinic.

The treatment modalities included chemotherapy only in 44% of patients and chemotherapy plus radiotherapy in 56% of patients. The median number of chemotherapy cycles completed was five (range 1-8 cycles). Intrathecal chemotherapy was administered to 24% of patients with central nervous system (CNS) involvement or patients at risk of CNS involvement. The majority of patients had chemotherapy delayed during their treatment due to severe neutropenia or complications from PTB or HIV treatment. For the 14 patients who received radiotherapy, half of them received IFRT (dose range 20Gy-40Gy) and the other half of patients received palliative radiotherapy (dose range 8Gy-30Gy) for progressive or relapsed disease.

Patients who completed the initial intended treatment were evaluated for treatment response. As shown in Table 3; 76% of HIV positive patients were evaluable. The remaining patients were either lost to follow up or died before completing the initial intended treatment with no record of the achieved response. Complete response was achieved in the majority of patients. A partial response was seen in one patient whereas 32% of patients progressed on treatment. For those patients who progressed on chemotherapy, three were offered palliative radiotherapy with doses ranging from 8 Gy to 20 Gy and one patient received palliative CMV

(Cyclophosphamide, Methotrexate and Vincristine) chemotherapy. Relapses were seen in only two (17%) patients at 12 weeks and 123 weeks after completion of treatment. The first patient relapsed at the initial primary site in the nasopharynx and brain and was subsequently treated with palliative CMV chemotherapy and palliative whole brain radiotherapy (WBRT) to 20Gy. The other patient's primary disease was in the liver, spleen and oral cavity and relapsed in the left cervical node and was subsequently offered palliative chemotherapy and died after one cycle of chemotherapy.

The Kaplan-Meier curves for overall survival and the impact of the analysed factors are shown in Figure 2 and Figure 3.

As shown in Figure 2 the estimated overall survival of HIV positive patients at 1 year and 3 years was 45% and 39% respectively.

At the time of analysis of this cohort, five (25%) patients were still known to be alive. These patients were more than five years post treatment. Only two of the surviving patients were still being followed up, the other three patients were lost to follow up. Records from the South African Department of Home Affairs however proved the three patients lost to follow up were still alive.

The following factors were analysed to evaluate their impact on overall survival: stage, IPI, CD4 count, chemotherapy alone vs. chemotherapy and radiotherapy, extranodal status and gender.

For the above factors stage I/II showed better OS than stage III/IV with 1 year OS of 63% and 23% respectively and 3 years OS of 50% and 11% respectively (p=0.045) as shown in Figure 3A. The IPI when comparing low risk disease with intermediate disease showed a 1 year OS of 58% and 25% respectively and 3 year OS of 58% and 13% respectively (p=0.040). The 1 year OS for patients treated with chemotherapy alone versus chemotherapy and radiotherapy were 18% and 57% respectively and 3 years OS were 0% and 56% respectively (p=0.021) in favour of chemotherapy followed by radiotherapy. This statistically significant p-value included patients treated with IFRT after complete response and palliative radiotherapy for partial response and progressive disease. Graph 3F shows that patients with low risk disease had better OS than patients with intermediate risk disease with a 1 year OS of 57% and 27% respectively and a 3 year OS of 57% and 13% respectively

(p=0.040). As shown in Graph 3E, there was a positive trend towards better OS for patients with CD4>200 cells/ml^3 compared to those with a CD4<200 cells/ml^3 although the p-value was not statistically significant (p=0.42). Gender did not impact on OS as shown in Figure 3D. The number of extranodal sites involved also did not show any statistically significant impact on OS (p=0.57), however for patients with only one extranodal site there was a positive trend after approximately two years of follow up.

Discussion

In this cohort we evaluated 28 patients who presented at Groote Schuur Hospital between January 2004 and December 2009 with a histological diagnosis of Plasmablastic Lymphoma. PBL is a variant of DLBCL which was recognised as a separate entity and described by Delecluse et al in 1997 [1]. This type of lymphoma is associated with HIV/AIDS infection.

South Africa has a high HIV/AIDS prevalence. According to the Midyear Population Estimates 2011 report by Statistics South Africa, the estimated overall HIV prevalence rate is 10.6% with a total of 5.38 million people living with HIV in 2011. An estimated 16.6% of the adult population aged 15-49 years of age is HIV positive [26]. With these high national HIV/AIDS prevalence rates a significant number of HIV related lymphomas like DLBCL, Burkitt's lymphoma and PBL are diagnosed [27,28] .

Despite the improvements in diagnosis by modern techniques including immunohistochemistry and other newer tools and the introduction of more intense chemotherapy regimens, the diagnosis and treatment of PBL still remains challenging. The overall survival rates and relapse rates still remain disappointingly poor.

This rare HIV related lymphoma makes up 2.6% of all HIV related Non-Hodgkin lymphomas. The true incidence is unknown due to its rarity and the lack of large trials [2,3]. Since this group was overwhelmingly dominated by HIV positive patients, the discussion will mainly focus in PBL in the setting of HIV. In our study the median age at presentation for HIV positive patients was 35 years which is consistent with other studies and case reports [16,29-32]. In these studies there was a slight male predominance. In our study there was slight female predominance. This observation is consistent with the HIV prevalence in South Africa which shows a female predominance. When looking at the HIV distribution by race in

our cohort, most of patients were black, a few of the patients were mixed race and only a minority of patients were whites. This is consistent with the national racial breakdown [26].

Patients living with HIV/AIDS are at an increased risk of developing HIV related lymphomas [33]. The pathogenesis of PBL is multifactorial. Although typically associated with HIV infection, Castillo and his colleagues reported on the largest known cohort of PBL in HIV negative patients. They noted distinct clinopathological characteristics such as short OS, lower rates of oral involvement and EBV association [34]. Most of their patients had other immunosuppressive conditions including autoimmune diseases and solid organ transplants, but in some cases no cause of immunosuppression was identified. The HIV negative patients in this study had no known immunosuppressive conditions and all died of lymphoma.

EBV has also been associated with pathogenesis. Several authors have reported more than 70% association with EBV [32,35-37]. In this study EBV was not tested for as it is not essential for diagnosis. The role played by EBV in the pathogenesis of HIV related lymphoproliferative disorders is still a subject of debate. It is believed that EBV encodes a series of products interacting with or exhibiting homology to a wide variety of antiapoptotic molecules, cytokines and signal transducers and therefore promoting EBV infection, immortalization and malignant transformation [37,38].

Plasmablastic lymphoma may be aetiologically related to plasmacytoma e.g. Qing and colleagues reported a case where PBL occurred as a result of transformation from a plasmacytoma [39]. This association of PBL with plasma cell myelomas was studied by a number of authors who demonstrated immunophenotypic similarities between PBL and plasma cell myeloma [18]. However, Chung Che and colleagues reported that PBL was more similar to DLBCL immunohistochemically than to plasma cell myeloma [17].

In the initial reports, PBL was described in the oral cavity and hence the name PBL of the oral cavity. New cases have been reported since 1997 which were considered pathologically to be PBL but occurring extraorally. These studies indicated that PBL is very heterogeneous and may occur in several extraoral sites including the gastrointestinal system, paranasal sinuses, nasopharynx, skin, central nervous system, lymph nodes, subcutaneous tissues and other organs. This led to the nomenclature being changed to plasmablastic lymphoma [32,38]. In this study there was significant heterogeneous site involvement including the oral

cavity, oropharynx, gastrointestinal and other sites rarely reported in the literature including kidney, liver, ascites, pleural effusion and mediastinum (Table 3).

In an editorial comment by Pantanowitz and Dezube (2007) entitled 'Plasmablastic Lymphoma, A diagnostic and Therapeutic Puzzle', the authors attempted to summarise the diagnostic and therapeutic challenges in PBL management. These challenges have not been met two decades after PBL was first described. The standard treatment has not been established to date although CHOP chemotherapy is widely accepted as first line therapy. The NCCN recommends more aggressive therapeutic regimens [25]. A number of other treatment options have been explored. Bibas and colleagues in a single institution experience used the proteozome inhibitor bortezomib which is indicated for the treatment of multiple myeloma alone or in combination with dexamethasone, oxaliplatin, gemcitabine, cytosine arabinose and pegfilgrastim (GOVDD). The authors reported that this was an effective and manageable therapeutic option in HIV associated PBL but they failed to show an overall survival benefit [40]. Other treatment options evaluated in the literature include the anti-CD20 monoclonal antibody Rituximab which showed a reasonable response in patients with minimal or partial CD20 expression [16,17]. There are no randomised controlled studies comparing standard CHOP chemotherapy with the more aggressive regimens. Single institutional experiences have not demonstrated any superiority of the more aggressive regimens to CHOP chemotherapy. The evidence for effectiveness of high dose salvage chemotherapy and stem cell transplant in PBL is scanty but since it was shown to be effective in DLBCL, some authors suggest that it may be a viable option in first remission [31,40,41]. These differences in opinions may exemplify the unique clinicopathologic features of PBL.

The impact of HAART on the treatment and prognosis of HIV related lymphomas has been an area of research and debate but it has now been accepted that HAART improves treatment outcomes in HIV/AIDS related lymphomas with even some reported cases of spontaneous regressions of PBL on HAART [42-45]. In this study all patients were commenced on HAART before chemotherapy was commenced and five patients are alive at more than five years since diagnosis of PBL. A third of patients were on HAART at presentation and the rest were initiated prior to commencement of chemotherapy unless there was a life threatening condition necessitating urgent commencement of chemotherapy e.g. airway compromise by tumour.

Standard CHOP was used in the majority of patients. More than 68% of the HIV positive group attained a complete or a good partial response to chemotherapy. This was comparable to results reported in other studies [31,32,34,45]. Most patients had low or intermediate risk disease according to the IPI and only a few patients had high risk disease. The treatment outcomes remain poor. The above studies also demonstrated that patients treated for PBL regardless of chemotherapy regimen used have been shown to achieve high response rates but tend to relapse very early from within a few weeks to a few months post treatment with most patients dying within the first year after diagnosis. Similarly most of our patients died within the first year after diagnosis. In this cohort only a minority of patients were documented to have relapsed after achieving complete response however most patients with documented complete response were lost to follow up. In the South African context it may be speculated that the poor outcomes may be attributed to severe immunosuppression, poor general condition at presentation, presence of other comorbidities including tuberculosis, possible drug interactions with tuberculosis treatment and treatment delays due to various reasons. More than 60% of the HIV-positive cohort had treatment delays with some patients experiencing multiple chemotherapy delays. These treatment delays were due to poor treatment tolerance, toxicity especially severe pancytopenia and poor compliance. During the period when these patients were treated, growth colony stimulating factors (GCSF) were not freely available at our institution.

In this cohort, the median OS for HIV positive patients was 13 months. In a study by Castillo and his colleagues entitled 'Prognostic factors in chemotherapy treated patients with HIV-associated lymphoma' their findings were a median OS of 14 months and a 5 year OS of 31% [31]. These results were similar and comparable to this cohort.

A study by Montes-Moreno in a population of HIV positive patients reported disease free survival of around 42% at five years [46]. These results, although not reproduced in other studies also show a pattern of early relapse and poor survival outcomes as observed in the current study.

Little is known about the potential impact of any prognostic factors on survival outcomes. In this cohort early stage and chemotherapy followed by radiotherapy demonstrated a positive impact on prognosis. This positive trend was also noted in radiotherapy given as palliation therefore signalling a potential role for palliative radiotherapy in the management of relapsed

or progressive disease. The IPI was a good predictor of outcome in the low risk disease versus intermediate disease but it may need to be adapted for HIV related PBL as the prognostic groupings appears to be different to that of DLBCL. The possible explanation to this positive impact on survival outcomes by these factors may be due the fact that these were early disease patients. These patients also received radiotherapy after completion of chemotherapy which may have impacted on the results.

Gender, number of extranodal sites involved and CD4 count did not affect survival outcomes. Patients who presented with a CD4 count of >200 cells/ml^3 however did show a trend towards better survival. The CD4 level of <200 cells/ml^3 was chosen because that was the value used to initiate HAART at the time that the patients were treated in this study. In other studies improved OS was associated with achieving a complete response and the use of concurrent HAART with chemotherapy. These factors were not evaluated in the current study. Similarly EBV status, sex, primary site of involvement, clinical stage, and CD4 count failed to show any association with survival in a series of other studies done by several other authors [38,39,45,47].

Conclusions

In this cohort of 28 patients treated for Plasmablastic Lymphoma between January 2004 and December 2009, the survival outcomes as well as the patient demographics and disease profiles were reviewed.

Slightly more than half of the patients presented with early disease. The population of patients in this cohort displayed a wide heterogeneous site involvement. Only a third of patients had B-symptoms at presentation and a tenth of the patients had bone marrow involvement.

At Groote Schuur Hospital CHOP chemotherapy remains the treatment of choice for PBL. The treatment outcomes reported in the current study are comparable with other studies. The patients in this study displayed high overall response rates but generally poor treatment outcomes as described in other studies. Patients who presented with early stage disease, low,

intermediate risk disease and who received radiotherapy after chemotherapy showed a better overall survival.

Several studies have failed to establish prognostic factors in PBL. In this study early disease presentation and the use of radiotherapy was shown to improve survival outcomes.

The diagnosis and treatment of PBL remains a challenge and therefore early diagnosis and treatment of PBL like other lymphomas is very crucial so as to achieve good survival rates. To further establish standard care guidelines large multi institutional randomised trials are required but due to the rarity of PBL, this may not be feasible.

Materials and Methods

Folders for patients treated for PBL between 2004 and 2009 were retrospectively reviewed. The list for these patients was obtained from the Groote Schuur Hospital Radiation Oncology Department. Ethics approval was obtained from the Health and Research Ethics Committee of the University of Cape Town and permission from the Clinical Director of Groote Schuur Hospital was obtained before commencement of the study. A literature review was performed using Pubmed, Google, available journals and text books.

All folders reviewed included only patients over the age of 18 years with a confirmed histological diagnosis of PBL. The data extracted was recorded into customised data sheets and transferred into an excel spreadsheet.

The data collected included age at diagnosis, date of presentation, gender, ethnic group, site of disease, stage of disease, ECOG performance status, lactate dehydrogenase (LDH) and the International Prognostic Index (IPI). The IPI was calculated by incorporating the following factors; age >60 years, serum lactate dehydrogenase (LDH) levels >1x normal, ECOG performance status of 2-4, stage III or IV and >1 extranodal site involvement. The presence of B-symptoms which included weight loss of >10% in the last 6 months, unexplained fever or night sweats was noted.

In addition to the above, HIV status, CD4 count and viral load when available were included in the data sheet as well as the presence of any infections like pulmonary tuberculosis (PTB),

varicella zoster and other malignancies like Kaposi sarcoma. Time on HAART at diagnosis was also recorded.

The treatment approach was documented including the chemotherapy regimen used, number of cycles completed and radiotherapy doses for those patients who received radiotherapy. Responses to treatment were recorded as complete response (CR), partial response (PR) and progressive disease (PD). For patients who achieved a complete response and relapsed, the time to relapse, site of relapse and the treatment offered for the relapse was documented.

Complete response was defined as the clinically or radiological disappearance of all evidence of disease. Partial response was described as regression of measurable disease with no new sites and progressive disease was defined as evidence of new lesions or more than 50% increase in previously involved sites from nadir [48].

At last follow up, the patients' status was recorded as either alive with disease, alive without disease or dead, and if recorded as dead it was noted whether they died from lymphoma or other causes.

At initial presentation, all the patients had a histological diagnosis of plasmablastic lymphoma. A detailed history and clinical examination was done. Staging investigations consisted of a biochemical and haematological profile including a full blood count, renal function tests, liver function tests and LDH levels. An HIV test was performed in those patients with unknown HIV status. A CT scan of the neck, chest abdomen and pelvis and a bone marrow aspiration and trephine (BMAT) were done. PET/CT was not utilised for staging in this study. Patients were staged using the Cotswold Modified Ann Arbor staging system.

All patients who were not yet on HAART at presentation were referred to the infectious disease unit for counselling and commencement of HAART.

The chemotherapy regimen used for all patients was CHOP chemotherapy initially with the intention to give five cycles followed by restaging investigations and to continue on chemotherapy depending on response. Intrathecal (IT) chemotherapy using methotrexate, cytosine arabinosine and dexamethasone was administered to patients at risk of central nervous spread e.g. brain or spinal cord involvement, paratesticular sites, parameningial sites

etc. Patients with pancytopenia prior to commencement of chemotherapy received Bleomycin, Vincristine and Prednisone (BOP) chemotherapy until pancytopenia resolved. Patients with a poor performance status received a reduced dose of CHOP until their clinical condition stabilised and one patient with cardiac related problems received etoposide, cytosine arabinosine and methyprednisolone. Some patients received Involved Field Radiotherapy (IFRT) or palliative radiotherapy as indicated as per treating physician discretion. Those who progressed on chemotherapy or relapsed were treated with palliative chemotherapy with or without palliative radiotherapy depending on the sites of disease. The palliative chemotherapy regimen used consisted of low dose Cyclophosphamide, Methotrexate and Vincristine (CMV).

HIV positive patients do not qualify for high dose salvage chemotherapy and stem cell transplant at our institution due to lack of resources. The HIV negative patient who progressed on CHOP received salvage therapy with DHAP (cisplatin, cytosine arabinosine and dexamethasone).

Patients who completed therapy were followed up 3 monthly for the first year then 4-6 monthly for three years and annually thereafter. The disease status was evaluated by history, clinical examinations, full blood count and blood chemistry especially LDH. Imaging, biopsy and any other investigations were only considered as per clinician's discretion and clinical findings.

Statistical analysis was performed using GraphPad Prism version 6 for Windows. Overall Survival was calculated from time of registration to time of death due to lymphoma or any other cause or date last seen for patients lost to follow up. Progression Free Survival (PFS) was calculated from time of registration to time of first progression on primary treatment, death or date last seen for those patients lost to follow up. Survival curves were generated using the Kaplan-Meier method and compared using the log rank test. A p value of <0.05 was considered statistically significant.

List of abbreviations

- CHOP: cyclophosphamide, adriamycin, vincristine and prednisone
- DHAP: cisplatin, cytosinarabinosine and dexamethasone
- IPI: International Prognostic Index
- OS: overall survival
- PFS: progression free survival
- LDH: lactate dehydrogenase
- CMV: cyclophosphamide, methotrexate and vincristine
- IFRT: involved field radiotherapy
- IT: intrathecal chemotherapy
- ECOG: Eastern Cooperative Oncology Group
- HAART: highly active antiretroviral therapy
- PBL: plasmablastic lymphoma
- HIV: Human Immunodeficiency Virus
- GSH: Groote Schuur Hospital

Competing interests

Dr Sebathu P Chiyapo declares no competing interests. No funding or fees were received in any form in the preparation of this manuscript

Dr Zainab Mohamed declares no competing interests. No funding or fees were received in any form in the preparation of this manuscript

Authors Contribution

Dr Sebathu P Chiyapo

Was responsible for the literature research, data analysis, write up of the manuscript

Dr Zainab Mohamed

Was responsible for supervision, data analysis and editing of the manuscript

Authors' information

Dr SPC is a radiation oncology resident at the Department of Radiation Oncology at Groote Schuur Hospital in Cape Town, South Africa. He holds an MB.BS from the University of the West Indies in Trinidad and Tobago and he is a Fellow of the College of Radiation Oncologist of South Africa.

Dr ZM is a Senior Specialist in the Radiation Oncology Department, Groote Schuur Hospital, and University of Cape Town. She obtained her undergraduate degree (MBChB) at the University of Cape Town in 1992 and her MMed in Radiation Oncology at the University of Stellenbosch in 2004. Her research interests are HIV-related cancers (lymphoma and Kaposi's sarcoma).

Acknowledgements

I am thankful to all people who supported me in this journey. I have successfully completed my research project.

- Firstly my wife Tebogo Chiyapo and my daughter Oaratwa Chiyapo for understanding and giving me support and all the help I needed and believed in me even during times when I did not really believe in myself.
- My supervisor Dr Zainab Mohamed for her valuable insight and support and guidance throughout the whole process
- Dr Alister Hunter for his input, guidance and support
- To all my radiation oncology colleagues and staff for all their support
- The staff at the filing office who ensured that I had all the patients folders that I needed to evaluate
- I am also thankful to my mother Ms Wapiwa Samson and my in-laws who gave me support and encouraged me to carry on

This journey would not have been easy if it was not for the support and dedication you all have shown to me. I pray to God the Almighty to give you all his blessings and keep you safe and blessed at all times.

Thank You!

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Tables and Figures

Table 1. Patient's demographics and disease characteristics

Profile	HIV +ve Patients n=25
Age Mean Median Range	 38 35 30-57
Sex Male Female	 12(48%) 13(52%)
Race Black White Coloured	 20(80%) 1(4%) 4 (16%)
Stage I/II III/IV Nodal only Extranodal only Extranodal + nodal 1 Extranodal site >1 Extranodal sites	 13(52%) 12(48%) 1(4%) 18(72%) 6(24%) 15(63%) 9(37%)
IPI Low risk 0-1 Intermediate 2-3 High risk 4-5	 14(56%) 9(36%) 2(8%)
B-symptoms +ve -ve	 8(32%) 17(68%)
CD4 count Median Range	 196 23-368
Treatment Chemotherapy alone Chemo + RT Median RT Dose RT dose Range	 11(44%) 14(56%) 30 20-40

Figure 1: Treatment Summary all patients

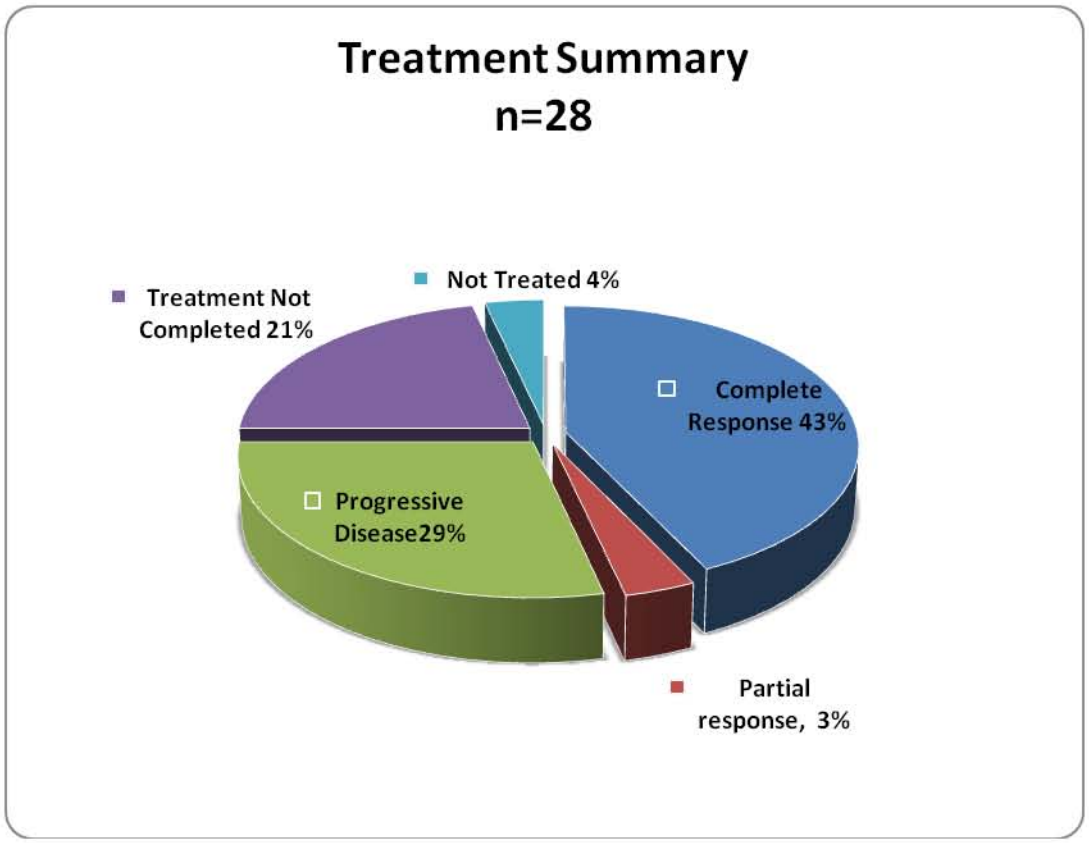


Table 2: Disease distribution by site in all patients

Site	Percentage of patients
Bone	**21%**
Oral cavity, oropharynx	**18%**
Nasopharynx and Sinuses	**18%**
Spleen	**14%**
Anorectum	**14%**
Bone Marrow	**11%**
Liver	**11%**
Exradural, Cerebrospinal fluid and Brain	**11%**
Lymph nodes	**11%**
scalp	**7%**
Mediastinum	**4%**
Skin and subcutaneous	**4%**
Kidney	**4%**
***Other**	**14%**

***pleural effusion, ascites, unspecified site**

Table 3: Response Rates (RR) in evaluable patients

	All patients **n=21**	**HIV +ve** **n=19**
CR	**12(57%)**	**12(63%)**
PR	**1(5%)**	**1(5%)**
PD	**8(38%)**	**6(32%)**

Figure 2. Overall Survival in all HIV Positive patients

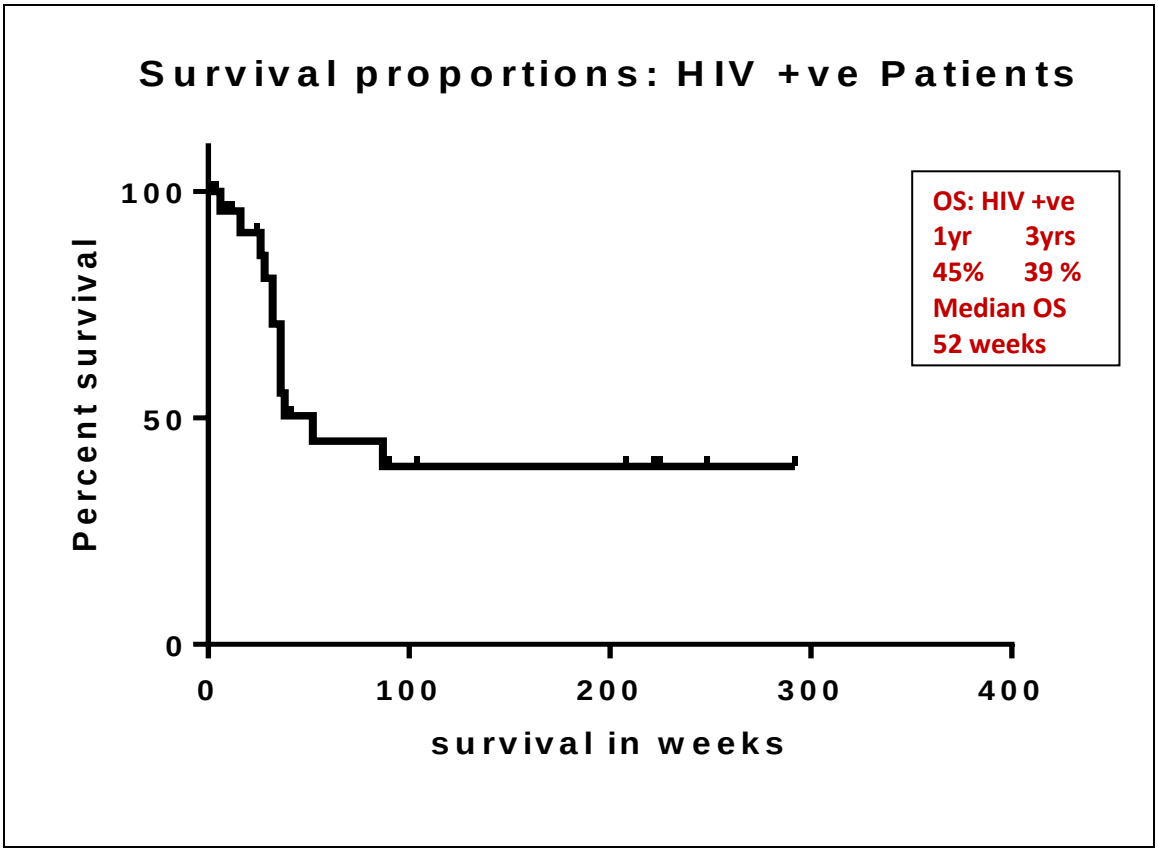
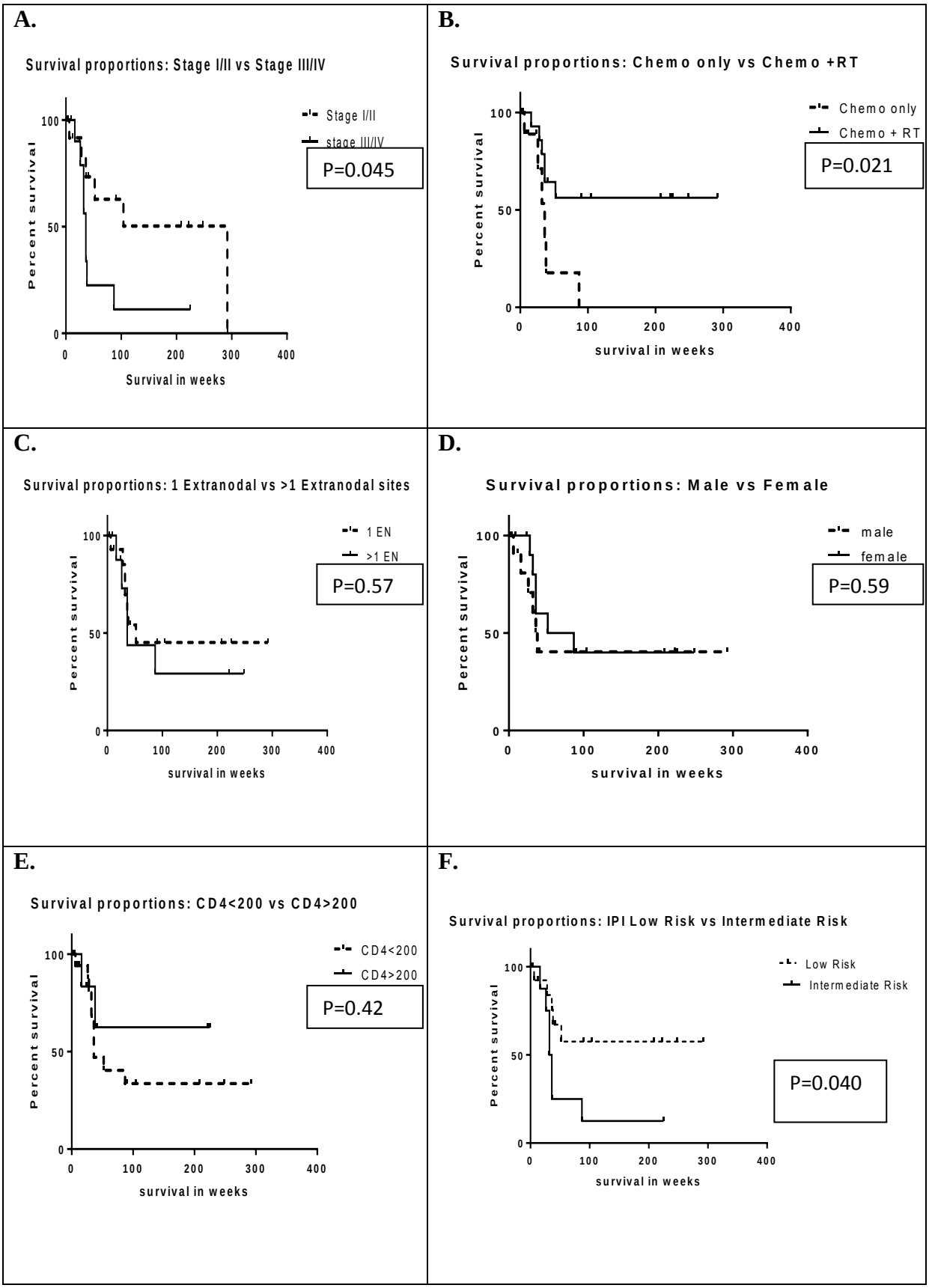


Figure 3: Survival curves comparing different factors and their impact on survival in HIV positive patients



PART D: APPENDICES

I. Data Capture Instrument

II. Official Ethics Approval Letters

III. Guidelines to Authors for Submission

I. Data Capture Instruments

DATA ENTRY FORM

1. **Patient Demographics**

Initials	
Date of Birth	
Id Number	
Hospital Number	
RT Number	
Race	
Age at presentation	
Date of Presentation	
Date of Commencement of Treatment	

2. Disease Site

3. **Tumor EBV status**

Positive	Negative	Unknown

5. HIV Status

Positive	Negative	Unknown

If Positive

CD4 count	Viral load

6. Stage

I	II	III	IV

A	
B	

7. Prognostic Factors

Age > 60	
Performance Status 2-4	
Extranodal Involvement Site	
Serum LDH>1.5x normal	
Stage III or IV disease	
IPI	

8.

Months since HIV diagnosis	
Months on ARV's at Diagnosis	

9. Opportunistic Infections

10. Other Malignancies

11. For HIV negative

Other medical conditions	**Medications**	**Duration**

12. Management

Chemotherapy		Radiotherapy	
Regimen		Site	
No of cycles		Type / Dose	

13. Restaging

Timing Of Restaging Investigations	

14. Completion of Intended Primary Treatment

Yes	
No	

If No, Why?

_____

_____

15. Response to primary treatment

CR	PR	SD	PD	Not Completed

16. Relapse

Yes	
No	

If Yes

Time to Relapse	Site of Relapse

17. Treatment of Relapse

	Salvage chemo	Radiotherapy	Palliation
Type			

Completion of Salvage Chemo

Yes	
No	

If No, why not?

_____

_____

18. Current Status

Alive and free of disease	
Alive with disease	
Lost to follow up	
Dead	

If dead, death due to:

Disease	
Treatment complications	
Other	
Unknown	

19. Disease Free Survival

Date of Treatment Start	Date of relapse	Date last seen

Time till Relapse: _____

20. Overall Survival

Date of Treatment Start	Date of Death	Date last seen

Time till Death: _____

Any other relevant information regarding this patient?_____
_____
_____

II: OFFICIAL ETHICS APPROVAL LETTERS:

Department of Radiation Oncology
Groote Schuur Hospital
Cape Town

16th March 2012

The Ethics Committee
Groote Schuur Hospital
Cape Town

Dear Sir/Madam

Request for Expedited Review of College Commentary / Mmed for Radiation Oncology.

I plan to do a retrospective review on patients treated in Groote Schuur Hospital between 2004 and 2009 for Plasmablastic Lymphoma in both HIV negative and HIV positive patients. My end points will be overall survival, disease free survival, relapse rates.

This will entail a retrospective review of patient records. Strict patient confidentiality will be maintained and there will be no disclosure of patient details. There will be no patient contact. Patients will remain anonymous.

I hereby request an expedited review of this college commentary/Mmed mini thesis for radiation oncology.

Yours sincerely,

Dr. Sebathu P Chiyapo

Radiation Oncology Department

NB. Dr Z. Mohamed had previously requested ethics approval to do the research project which was approved on 22 November 2010, the approval expired on 30th November 2011 and the reference number was HREC: 555/2010

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

16 April 2012

HREC REF: 181/2012

Dr S Chiyapo
c/o Dr Z Mohamed
Radiation Oncology
LE32

Dear Dr Chiyapo

PROJECT TITLE: RETROSPECTIVE STUDY OF PATIENTS TREATED FOR PLASMABLASTIC LYMPHOMA BETWEEN 2004 AND 2009 IN GROOTE SCHUUR HOSPITAL.

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th April 2013.

Please submit a progress form, using the standardised Annual Report Form (FHS017), if the study continues beyond the approval period. Please submit a Standard Closure form (FHS019) if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP ICH, 135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s.thomas

Enquiries : Dr. Sebathu. P Chiyapo
Telephone : (021) – 404-4276
Fax :
E-mail : sebathuspc@hotmail.com
Reference :
Date : 19th April 2012

Departement van Gesondheid
Department of Health
ISebe IezeMoilo

Dr. B. Patel
Chief Medical Superintendent
Clinical Directorate
G45, OMB
Groote Schuur Hospital
Observatory
7925

Dear Dr Patel,

- Re: Audit project: *"A Retrospective Study of Patients Treated for Plasmablastic Lymphoma Between 2004 and 2009 in Groote Schuur Hospital"*

I hereby wish to apply for permission to perform an audit study, of the above title, in the Radiation Oncology Department of this hospital. It is a requirement of the MMed degree/ College exam (FRC Rad Onc.)

This project will involve an audit of radiotherapy folders only; patients will not be contacted or interviewed.

Our Departmental Research Committee has approved the study, as well as the Research Ethics Committee. The HREC. REF is 181/2012. I have included a copy of my study proposal and ethics approval letter.

I shall therefore be grateful to receive your approval, as the member of the Clinical Directorate responsible for research projects at Groote Schuur Hospital, to proceed with this study. Please let me know if any other documentation is required

Thank you

Sincerely

Dr Sebathu P Chiyapo

(Registrar,
Dept Radiation Oncology)

LE 32 Clinics

Western Cape Government
Health

DIRECTORATE: HEALTH
Groote Schuur Hospital
Enquiries: Dr Bhavna Patel
E-mail : Bhavna.Patel@pgwc.gov.za

Dr S. P. Chiyapo
Registrar
Department: Radiation Oncology
New Main Building

E-mail: sebathuspc@hotmail.com

Dear Dr Chiyapo

RESEARCH AUDIT PROJECT: A Retrospective Study of Patients Treated for Plasmablastic Lymphoma Between 2004 and 2009 in Groote Schuur Hospital

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research.

Please note the following:

a) Your research may not interfere with normal patient care
b) Hospital staff may not be asked to assist with the research.
c) No hospital consumables and stationary may be used.
d) No patient folders may be removed from the premises or be inaccessible. Please liaise with Mr Noel Weeder on ext. 4058 or 4066 in this regard.
e) Please introduce yourself to the person in charge of an area before commencing.
f) Please discuss the study with the Head of Radiation Oncology before commencing.

I would like to wish you every success with the project.

Yours sincerely

Signed by candidate

DR BHAVNA PATEL
SENIOR MANAGER: MEDICAL SERVICES
Date: 15th May 2012

G46 Management Suite, Old Main Building,
Observatory 7925

Private Bag X,
Observatory, 7935

HUMAN RESEARCH ETHICS COMMITTEE
-2 MAY 2013
HEALTH SCIENCES FACULTY

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.4.2014
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate	Date Signed	2/5/13

Principal Investigator to complete the following:

1. Protocol Information

Date form submitted	29/04/13		
HREC REF Number	181/2012	Current Ethics Approval was granted until	30th April 2013
Protocol title	Retrospective study of patient treated for Plasmablastic lymphoma between 2004 and 2009 in Groote Schuur Hospital		
Protocol number (if applicable)			
Principal Investigator	Dr S.P. Chiyapo		
Department / Office Internal Mail Address	L232, Department of Radiation Oncology, Groote Schuur Hospital.		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.2 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☐ No

2. List of documentation

HUMAN RESEARCH ETHICS COMMITTEE
- 2 MAY 2013
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
☑ Approved	Type of review: Expedited	☐ Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature Chairperson of the HREC	Signed by candidate	Date 2/5/13

Note: All amendments should include a Synopsis justifying the changes for the amendment (please see notice dated 23 April 2012)

Principal Investigator to complete the following:

1. Protocol information

Date	26/04/13	
HREC REF Number	181/2012	
Protocol title	Retrospective study of patients treated for Plasmablastic lymphoma between 2004 and 2009 in Groote Schuur Hospital	
Protocol number (if applicable)		
Principal Investigator	Dr Sebothm P. Chiloane	
Department / Office Internal Mail Address	L6B2 Clinic, Department of Radiation Oncology, Groote Schuur Hospital.	
1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.2 Is this a major or a minor amendment? (see FHS009(b))	☐ Major	☑ Minor

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval.
This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.

The Project title changed from.

Retrospective study of patients treated for Plasmablastic lymphoma between 2004 ~~to~~ and 2009 in Groote Schuur Hospital. to.

Retrospective study of Patients treated for Plasmablastic lymphoma at Groote Schuur Hospital between 2004 and 2009.

II. Guidelines to Authors for Submission to the Journal of Hematology & Oncology

Instructions for authors

Research Articles

Submission process | Preparing main manuscript text | Preparing illustrations and figures | Preparing tables | Preparing additional files | Style and language

See 'About this journal' for descriptions of different article types and information about policies and the refereeing process.

Submission process

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review.

Please note that *Journal of Hematology & Oncology* levies an article-processing charge on all accepted Research Articles; if the submitting author's institution is a BioMed Central member the cost of the article-processing charge may be covered by the membership (see About page for detail). Please note that the membership is only automatically recognised on submission if the submitting author is based at the member institution.

To facilitate rapid publication and to minimize administrative costs, *Journal of Hematology & Oncology* prefers online submission.

Files can be submitted as a batch, or one by one. The submission process can be interrupted at any time; when users return to the site, they can carry on where they left off.

See below for examples of word processor and graphics file formats that can be accepted for the main manuscript document by the online submission system. Additional files of any type, such as movies, animations, or original data files, can also be submitted as part of the manuscript.

During submission you will be asked to provide a cover letter. Use this to explain why your manuscript should be published in the journal, to elaborate on any issues relating to our editorial policies in the 'About *Journal of Hematology & Oncology*' page, and to declare any potential competing interests. You will be also asked to provide the contact details (including email addresses) of potential peer reviewers for your manuscript. These should be experts in their field, who will be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same research institution. Suggested reviewers will be considered alongside potential reviewers recommended by the Editor-in-Chief and/or Editorial Board members.

Assistance with the process of manuscript preparation and submission is available from BioMed Central customer support team.

We also provide a collection of links to useful tools and resources for scientific authors on our Useful Tools page.

File formats

The following word processor file formats are acceptable for the main manuscript document:

- Microsoft word (DOC, DOCX)
- Rich text format (RTF)
- Portable document format (PDF)
- TeX/LaTeX (use BioMed Central's TeX template)
- DeVice Independent format (DVI)

TeX/LaTeX users: Please use BioMed Central's TeX template and BibTeX stylefile if you use TeX format. During the TeX submission process, please submit your TeX file as the main manuscript file and your bib/bbl file as a dependent file. Please also convert your TeX file into a PDF and submit this PDF as an additional file with the name 'Reference PDF'. This PDF will be used by internal staff as a reference point to check the layout of the article as the author intended. Please also note that all figures must be coded at the end of the TeX file and not inline.

If you have used another template for your manuscript, or if you do not wish to use BibTeX, then please submit your manuscript as a DVI file. We do not recommend converting to RTF.

For all TeX submissions, all relevant editable source must be submitted during the submission process. Failing to submit these source files will cause unnecessary delays in the publication procedures.

Publishing Datasets

Through a special arrangement with LabArchives, LLC, authors submitting manuscripts to Journal of Hematology & Oncology can obtain a complimentary subscription to LabArchives with an allotment of 100MB of storage. LabArchives is an Electronic Laboratory Notebook which will enable scientists to share and publish data files in situ; you can then link your paper to these data. Data files linked to published articles are assigned digital object identifiers (DOIs) and will remain available in perpetuity. Use of LabArchives or similar data publishing services does not replace preexisting data deposition requirements, such as for nucleic acid sequences, protein sequences and atomic coordinates.

Instructions on assigning DOIs to datasets, so they can be permanently linked to publications, can be found on the LabArchives website. Use of LabArchives' software has no influence on the editorial decision to accept or reject a manuscript.

Authors linking datasets to their publications should include an Availability of supporting data section in their manuscript and cite the dataset in their reference list.

Preparing main manuscript text

General guidelines of the journal's style and language are given below.

Overview of manuscript sections for Research Articles

Manuscripts for Research Articles submitted to *Journal of Hematology & Oncology* should be divided into the following sections (in this order):

- Title page
- Abstract
- Additional non-English language abstract
- Keywords
- Background
- Results and discussion
- Conclusions
- Methods
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
- Tables and captions
- Preparing additional files

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

You can download a template (Mac and Windows compatible; Microsoft Word 98/2000) for your article.

For reporting standards please see the information in the About section.

Title page

The title page should:

- provide the title of the article
- list the full names, institutional addresses and email addresses for all authors
- indicate the corresponding author

Please note:

- the title should include the study design, for example "A versus B in the treatment of C: a randomized controlled trial X is a risk factor for Y: a case control study"
- abbreviations within the title should be avoided

Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background,** the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

Additional non-English language abstract

An additional non-English language abstract can be included within the article. The additional abstract should be placed after the official English language abstract in the submitted manuscript file and should not exceed 350 words. Please ensure you indicate the language of your abstract. In addition to English, we can support German, Spanish, French, Norwegian and Portuguese abstracts.

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. Reports of clinical research should, where appropriate, include a summary of a search of the literature to indicate why this study was necessary and what it aimed to contribute to the field. The section should end with a brief statement of what is being reported in the article.

Results and discussion

The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

Methods

The methods section should include the design of the study, the type of materials involved, a clear description of all comparisons, and the type of analysis used, to enable replication.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

Competing interests

A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

Authors are required to complete a declaration of competing interests. All competing interests that are declared will be listed at the end of published articles. Where an author gives no competing interests, the listing will read 'The author(s) declare that they have no competing interests'.

When completing your declaration, please consider the following questions:

Financial competing interests

- In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this

manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify.

- Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.
- Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.
- Do you have any other financial competing interests? If so, please specify.

Non-financial competing interests

Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

If you are unsure as to whether you, or one your co-authors, has a competing interest please discuss it with the editorial office.

Authors' contributions

In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section.

An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support.

Authors' information

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

The role of a scientific (medical) writer must be included in the acknowledgements section, including their source(s) of funding. We suggest wording such as 'We thank Jane Doe who provided medical writing services on behalf of XYZ Pharmaceuticals Ltd.'

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

Endnotes

Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

References

All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

Only articles, datasets, clinical trial registration records and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited; unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote

personal communications and unpublished data from the cited colleagues is the responsibility of the author. Footnotes are not allowed, but endnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE. Citations in the reference list should include all named authors, up to the first 30 before adding '*et al.*'..

Any *in press* articles cited within the references and necessary for the reviewers' assessment of the manuscript should be made available if requested by the editorial office.

Style files are available for use with popular bibliographic management software:

- BibTeX
- EndNote style file
- Reference Manager
- Zotero

Examples of the *Journal of Hematology & Oncology* reference style are shown below. Please ensure that the reference style is followed precisely; if the references are not in the correct style they may have to be retyped and carefully proofread.

All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, in the following format: **The Mouse Tumor Biology Database** [http://tumor.informatics.jax.org/mtbwi/index.do]. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Examples of the *Journal of Hematology & Oncology* reference style

Article within a journal
Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996, **13:**266-267.

Article within a journal supplement
Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999, **43**(Suppl 3):149-170.

In press article
Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract
Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings
Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book
Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal
Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings
Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book
Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series
Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.* Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology,* vol 1.]

Book with institutional author
Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis
Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL
The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)
Corpas M: **The Crowdfunding Genome Project: a personal genomics community with open source values** [http://blogs.biomedcentral.com/bmcblog/2012/07/16/the-crowdfunding-genome-project-a-personal-genomics-community-with-open-source-values/]

Dataset with persistent identifier
Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): **Genome data from sweet and grain sorghum (Sorghum bicolor)**. *GigaScience*. http://dx.doi.org/10.5524/100012.

Clinical trial registration record with persistent identifier
Mendelow, AD (2006): **Surgical Trial in Lobar Intracerebral Haemorrhage**. Current Controlled Trials. http://dx.doi.org/10.1186/ISRCTN22153967

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Illustrations should be provided as separate files, not embedded in the text file. Each figure should include a single illustration and should fit on a single page in portrait format. If a figure consists of separate parts, it is important that a single composite illustration file be submitted which contains all parts of the figure. There is no charge for the use of color figures.

Please read our figure preparation guidelines for detailed instructions on maximising the quality of your figures.

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- PPTX/PPT (single slide only)
- EPS
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- JPEG
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Figure legends

The legends should be included in the main manuscript text file at the end of the document, rather than being a part of the figure file. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals - i.e. Figure 1, 2, 3 etc); short title of figure (maximum 15 words); detailed legend, up to 300 words.

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If you wish to do so, you may submit an image which, in the event of publication, will be used to create a cover page for the PDF version of your article. The cover page will also display the journal logo, article title and citation details. The image may either be a figure from your manuscript or another relevant image. You must have permission from the copyright to reproduce the image. Images that do not meet our requirements will not be used.

Images must be 300dpi and 155mm square (1831 x 1831 pixels for a raster image).

Allowable formats - EPS, PDF (for line drawings), PNG, TIFF (for photographs and screen dumps), JPEG, BMP, DOC, PPT, CDX, TGF (ISIS/Draw).

Preparing tables

Each table should be numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title (above the table) that summarizes the whole table; it should be no longer than 15 words. Detailed legends may then follow, but they should be concise. Tables should always be cited in text in consecutive numerical order.

Smaller tables considered to be integral to the manuscript can be pasted into the end of the document text file, in A4 portrait or landscape format. These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review; this will not always be the case if columns are generated by simply using tabs to separate text. Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell display as black lines. Commas should not be used to indicate numerical values. Color and shading may not be used; parts of the table can be highlighted using symbols or bold text, the meaning of which should be explained in a table legend. Tables should not be embedded as figures or spreadsheet files.

Larger datasets or tables too wide for a landscape page can be uploaded separately as additional files. Additional files will not be displayed in the final, laid-out PDF of the article, but a link will be provided to the files as supplied by the author.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). As with all files, please use the standard file extensions.

Preparing additional files

Although *Journal of Hematology & Oncology* does not restrict the length and quantity of data included in an article, we encourage authors to provide datasets, tables, movies, or other information as additional files.

Please note: All Additional files **will be published** along with the article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files should be sent by email to editor@jhoonline.org, quoting the Manuscript ID number.

Results that would otherwise be indicated as "data not shown" can and should be included as additional files. Since many weblinks and URLs rapidly become broken, *Journal of Hematology & Oncology* requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.

Additional files can be in any format, and will be downloadable from the final published article as supplied by the author. We recommend CSV rather than PDF for tabular data.

Certain supported files formats are recognized and can be displayed to the user in the browser. These include most movie formats (for users with the Quicktime plugin), mini-websites prepared according to our guidelines, chemical structure files (MOL, PDB), geographic data files (KML).

If additional material is provided, please list the following information in a separate section of the manuscript text:

- File name (e.g. Additional file 1)
- File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

Additional file formats

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

- Additional documentation
    - PDF (Adode Acrobat)
- Animations
    - SWF (Shockwave Flash)
- Movies
    - MP4 (MPEG 4)
    - MOV (Quicktime)
- Tabular data
    - XLS, XLSX (Excel Spreadsheet)
    - CSV (Comma separated values)

As with figure files, files should be given the standard file extensions.

Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root.
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders.
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

Style and language

General

Currently, *Journal of Hematology & Oncology* can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise.

Journal of Hematology & Oncology will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on Writing titles and abstracts for scientific articles.

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. American Scientist also provides a list of resources for science writing. For more detailed guidance on preparing a manuscript and writing in English, please visit the BioMed Central author academy.

Abbreviations

Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the *Journal of Hematology & Oncology* reference format.
- Footnotes are not allowed, but endnotes are permitted.
- Please do not format the text in multiple columns.
- Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. **Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.**

Units

SI units should be used throughout (liter and molar are permitted, however).